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Human calprotectin: effect of calcium and zinc on its secondary and tertiary structures, and role of pH in its thermal stability.

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Abstract

Calprotectin, a heterodimeric complex belonging to the S100 protein family, has been found predominantly in the cytosolic fraction of neutrophils. In the present study, human calprotectin was purified from neutrophils using two-step ion exchange chromatography. The purified protein was used for circular dichroism study and fluorescence analysis in the presence of calcium and zinc at physiological concentrations, as well as for assessment of its inhibitory activity on the K562 leukemia cell line. The thermal stability of the protein at pH 7.0 (physiological pH) and 8.0 (similar to intestinal pH) was also compared. The results of cell proliferation analysis revealed that human calprotectin initiated growth inhibition of the tumor cells in a dose-dependent manner. The intrinsic fluorescence emission spectra of human calprotectin (50 microg/ml) in the presence of calcium and zinc ions show a reduction in fluorescence intensity, reflecting a conformational change within the protein with exposure of aromatic residues to the protein surface that is important for the biological function of calprotectin. The far ultraviolet-circular dichroism spectra of human calprotectin in the presence of calcium and zinc ions at physiological concentrations show a decrease in the alpha-helical content of the protein and an increase in beta- and other structures. Our results also show that increasing the pH level from 7.0 to 8.0 leads to a marked elevation in the thermal stability of human calprotectin, indicating a significant role for pH in the stability of calprotectin in the gut.

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